

Morphological and molecular data reveal one new species of the genus *Xenophrys* Günther, 1864 (Anura, Megophryidae) from Yunnan, China

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Abstract

Tongbiguan Provincial Nature Reserve, located in the western part of China's Yunnan Province adjoining northern Myanmar, harbors a striking diversity of species. Previous studies have indicated that herpetological diversity in this region remains underestimated. During herpetological surveys in 2024, five specimens of *Xenophrys* were collected from Tongbiguan Provincial Nature Reserve. Subsequent morphological comparisons and phylogenetic reconstruction indicate the presence of a distinct and previously unknown lineage within the genus *Xenophrys*, which we herein describe as a new species, *Xenophrys tongbiguanensis* **sp. nov.** This discovery brings the total number of recognized species in the genus to 32, including 13 in China and five in Tongbiguan Provincial Nature Reserve. The identification of this species further underscores the underestimated amphibian diversity of the reserve. Given its location along the China–Myanmar border, it is necessary to strengthen international cooperation in order to clarify the distribution of species within this transboundary region.

Key Words

Diversity, new species, Tongbiguan Provincial Nature Reserve, *Xenophrys*, *Xenophrys tongbiguanensis* sp. nov.

Introduction

The subfamily *Megophryinae* (Bonaparte, 1850) is widely distributed across tropical and subtropical Asia, including southern China, Indochina, the Malay Archipelago, and westward to Nepal and northeastern India (Lyu et al. 2023; Frost 2025). It currently comprises 142 recognized species, with over half described in the last ten years (Frost 2025).

The classification of *Megophryinae* has undergone several revisions in recent years (e.g., Chen et al. 2017; Mahony et al. 2017; Lyu et al. 2023). Most recently, Lyu et al. (2023) conducted a comprehensive review of the literature along with morphological and molecular phylogenetic analyses, proposing a new classification system recognizing ten genera. In this study, we follow the classification system of Frost (2025) and Lyu et al. (2023) for consistency in comparisons.

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Among these genera, *Xenophrys* Günther, 1864, is represented by 31 valid species distributed across Nepal, Bhutan, northeastern India, Bangladesh, Myanmar, Thailand, Laos, Vietnam, Cambodia, and southern China, with eleven species recorded from China (Frost 2025). Nearly one-third of the species in this genus have been described in the last five years (e.g., Luong et al. 2022; Saikia et al. 2024; Wu et al. 2024; Frost 2025). In addition, Wu et al. (2024) reported an undescribed lineage collected from Tongbiguan Nature Reserve, Yunnan, China, suggesting that additional cryptic species may exist within *Xenophrys*.

During herpetological surveys conducted in Tongbiguan Provincial Nature Reserve in 2024, five specimens of *Xenophrys* were collected. Subsequent analyses incorporating morphological characteristics and molecular data revealed that these specimens differ from known species of the genus. Herein, we describe them as a new species.

Materials and methods

Sampling

A total of five specimens were collected from Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan Province, China (Fig. 1), in August 2024. After photographs were taken, the frogs were euthanized using benzo-

caine. Liver tissues were sampled and stored in 95% ethanol for DNA extraction. The specimens were fixed in 10% formalin and later transferred to 75% ethanol for long-term storage. All specimens were deposited in the herpetological collection of the Museum of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS). Research protocols were approved by the Ethics Committee of the Kunming Institute of Zoology, Chinese Academy of Sciences (IACUC no.: IACUC-OE-2021-07-001).

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted using a phenol–chloroform extraction protocol (Sambrook et al. 1989). The mitochondrial 16S ribosomal RNA gene (16S rRNA) was amplified and sequenced using the primer pairs (5'–3'): 16SAR (CGCCTGTTTAYCAAAAACAT) and 16SBR (CCGGTYTGAAGTCAGATCAYGT) (Kocher et al. 1989). PCR amplifications were performed in a 25 µL reaction volume under the following conditions: an initial denaturation at 95 °C for 4 min; 36 cycles of denaturation at 95 °C for 40 s, annealing at 55 °C for 40 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. PCR products were purified using the Gel Extraction Mini Kit (Watson BioTechnologies, Shanghai, China) and

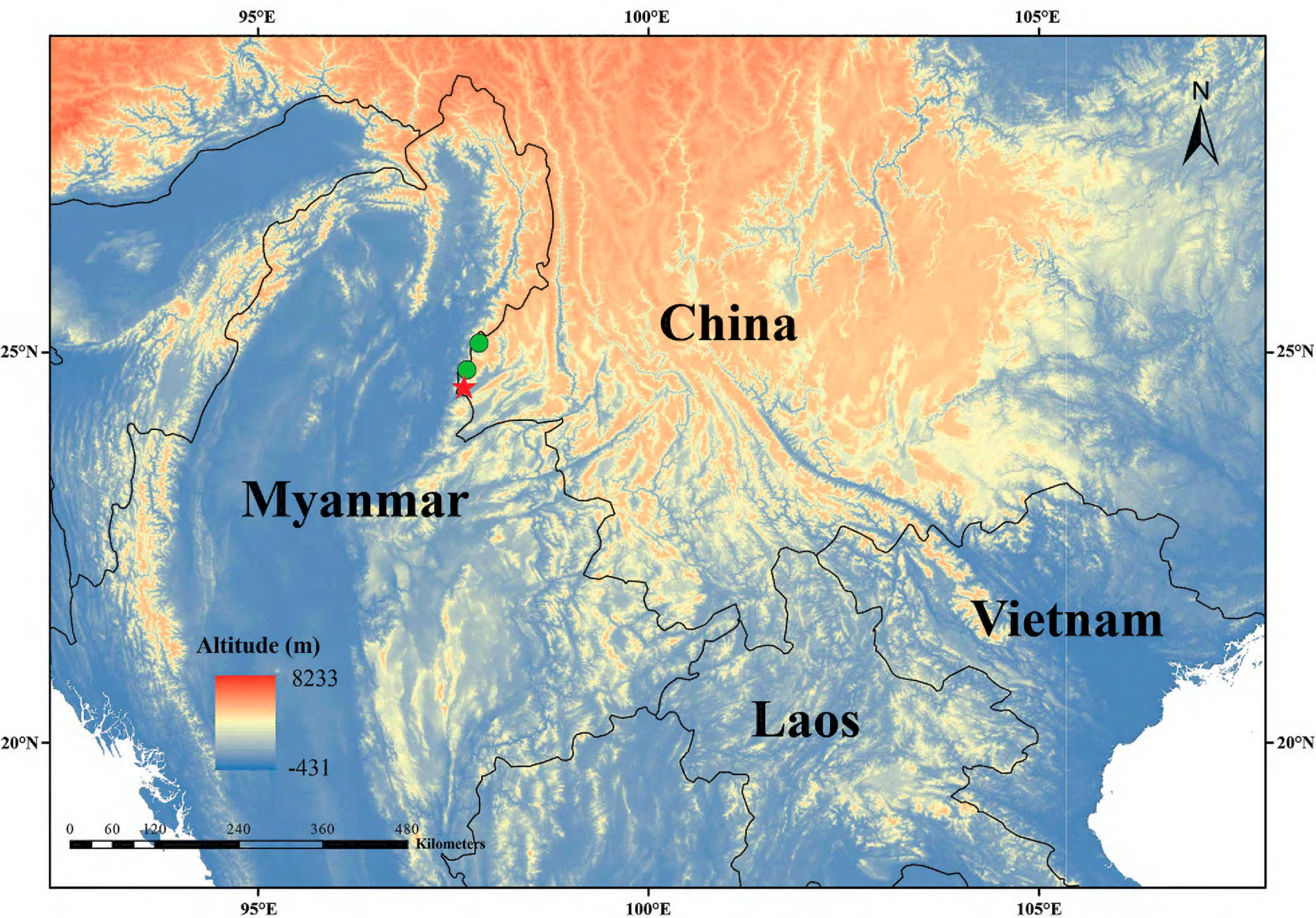


Figure 1. Known distribution of *Xenophrys tongbiguanensis* sp. nov. The red pentagram indicates the type locality of *Xenophrys tongbiguanensis* sp. nov.

sequenced with the same forward and reverse primers used in PCR. Sequencing was conducted using an ABI3730 automated DNA sequencer (Sangon Biotechnologies Co., Ltd., Shanghai, China). All sequences were assembled from forward and reverse reads and manually edited using AutoSeqMan (Sun 2018). New sequences were deposited in GenBank (for accession numbers, see Table 1).

Phylogenetic analysis

Phylogenetic analyses were conducted using the maximum likelihood (ML) and Bayesian inference (BI) methods. Seventy-two homologous sequences of *Xenophrys* and representative outgroups (*Brachytarsophrys feae*, *Leptobrachella ventripunctata*, and *Leptobrachium huashen*) were downloaded from GenBank (Table 1). The dataset was aligned using MUSCLE v3.8 (Edgar 2004), checked manually, and trimmed to minimize missing characters in MEGA v6.0.6 (Tamura et al. 2013). For the BI analyses, the best evolutionary model was selected based on the Bayesian Information Criterion (BIC; Posada 2008) using jModelTest v2.1.7 (Darriba et al. 2012). The GTR+G+I model was chosen as the best-fit model for 16S. The BI analysis was performed on CIPRES Science Gateway (Miller et al. 2010) using MrBayes v3.2.4 (Ronquist et al. 2012). Four Markov chains were run for 10 million generations, with sampling every 1,000 generations. The first 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to construct a consensus tree and estimate Bayesian posterior probabilities (BPPs). Convergence was assessed based on the average standard deviation of split frequencies (below 0.01) and effective sample size (ESS) values (greater than 200) in TRACER v1.5 (Rambaut and Drummond 2009). Maximum likelihood analyses were performed using RAxML with 1,000 bootstrap replicates under the rapid bootstrap algorithm (random seed value: 12,345), applying the GTR+I+G substitution model for the concatenated dataset (Stamatakis 2014). Mean genetic distances within and between species were calculated using uncorrected p-distances for 16S, implemented in MEGA v6.0.6 with complete deletion of gaps and missing data (Tamura et al. 2013).

Morphology and morphometrics

All measurements were taken with digital calipers to the nearest 0.1 mm. Morphological terminology followed Fei et al. (2009). Twenty-one measurements included the following: snout-vent length (SVL): measured from tip of snout to vent; head length (HDL): measured from tip of snout to rear of jaw; maximum head width (HDW): measured width of head at its widest point; snout length (SNT): measured from tip of snout to anterior corner of ocular aperture; eye diameter (ED): diameter of exposed portion of eyeball; tympanum diameter (TD): measured as maximal diameter of tympanum; width of upper eyelid (UEW):

maximum width of upper eyelid; distance from nostril to eye (DNE): distance from the front of the eye to the center of the nostril; distance from the center of the nostril to the tip of the snout (SN); internarial distance (IND): distance between nares; distance from anterior edge of tympanum to posterior corner of eye (TEY); interorbital distance (IOD): measured at narrowest point between eyes on top of the head; forearm length (FAL): measured from the elbow to the wrist; diameter of lower arm (LAD); forearm and hand length (FHL): distance from elbow to the tip of the third finger; tibia length (TL): distance from knee to heel; hand length (HL): distance from the posterior end of the inner metacarpal tubercle to tip of third finger; hindlimb length (HLL); foot length (FL): distance from the proximal end of inner metatarsal tubercle to the tip of fourth toe; thigh length (THL): from the cloaca to the knee; tarsus length (TAL): measured as the distance from knee to heel.

Results

The aligned sequence matrix of the 16S rRNA gene contained 489 bp, with 273 conserved sites and 215 variable sites. Of the variable sites in the alignment, 164 were parsimony-informative (including the outgroup sequences). The BI and ML analyses obtained essentially similar topologies with relatively high nodal support values for most terminal nodes, differing mainly at nodes identified as weakly supported or collapsed (Fig. 2). As shown in the tree (Fig. 2), the genus *Xenophrys* was recovered as monophyletic with strong support from both analyses (BPP = 1; BS = 97). The newly collected samples from Tongbiguan Nature Reserve, Yunnan, China, and samples proposed as *Xenophrys* sp. in Wu et al. (2024) clustered into a monophyletic clade with strong nodal support (BPP = 1; BS = 100). In addition, this clade clustered with *X. awuh*, *X. zunhebotensis*, *X. serchhipii*, *X. numhbumaeng*, *X. oropedion*, *X. ancrae*, *X. megacephala*, and *X. dzukou*, but with low support value.

The genetic distances based on the 16S gene among species of *Xenophrys* are presented in Table 2. The putative new species from Tongbiguan Provincial Nature Reserve showed obvious genetic divergence from other congeners. The genetic distance between the new populations and the other congeneric species ranged from 6.7% (with *X. ancrae*) to 11.7% (with *X. auralensis*, *X. robusta*, and *X. oreocrypta*). It is comparable to the divergences among the nearest neighbor genetic distances of the described *Xenophrys* species, which ranged from 2.1% (between *X. mangshanensis* and *X. maosonensis*) to 14.2% (between *X. awuh* and *X. oreocrypta*). In addition, these levels of pairwise divergence of the 16S rRNA gene exceeded the acceptable threshold (3%) of species-level genetic divergence in anurans (Fouquet et al. 2007).

Based on the results of the morphological examination presented below and phylogenetic analysis, the specimens from Tongbiguan Provincial Nature Reserve are regarded as a new species described herein.

Table 1. Localities, voucher information, and GenBank accession numbers for all specimens used in this study.

Species	Voucher	Locality	Accession No.	Reference
<i>Xenophrys ancrae</i>	SDBDU 2009.727	India, Arunachal, Changlang	KY022318	Mahony et al. 2018
<i>Xenophrys ancrae</i>	ZSI A 11606	India, Arunachal, Changlang	MN734391	Mahony et al. 2020
<i>Xenophrys auralensis</i>	NCSM 79599	Cambodia, Kampong Speu, Aural	KX811807	Chen et al. 2017
<i>Xenophrys awuh</i>	SDBDU 2007.192	India, Nagaland state, Kohima district, above New Ministers' Hill, Aradurah Forest	MN734399	Mahony et al. 2020
<i>Xenophrys awuh</i>	MZUHC 314	India, Mizoram	MT793046	Lalronunga et al. 2020
<i>Xenophrys dehongensis</i>	SYS a005823	China, Yunnan, Yingjiang	OQ180993	Lyu et al. 2023
<i>Xenophrys dehongensis</i>	KIZ 053847	China, Yunnan, Tongbiguan	PP989323	Wu et al. 2024
<i>Xenophrys dzukou</i>	SDBDU 2007.106	India, Nagaland, Kohima	KY022324	Mahony et al. 2017
<i>Xenophrys flavipunctata</i>	SDBDU 2009.298	India, East Khasi Hills district, Meghalaya	MH647517	Mahony et al. 2018
<i>Xenophrys flavipunctata</i>	SDBDU 2007.134	India, Kohima district, Nagaland	MH647518	Mahony et al. 2018
<i>Xenophrys glandulosa</i>	KIZ 013609	China, Yunnan, Wenlong	KX811761	Chen et al. 2017
<i>Xenophrys glandulosa</i>	SYS a003757	China, Yunnan, Mt Gaoligong	MH406754	Liu et al. 2018
<i>Xenophrys glandulosa</i>	KIZ 053845	China, Yunnan, Tongbiguan	PP989322	Wu et al. 2024
<i>Xenophrys himalayana</i>	SDBDU 2009.1227	India, West Kameng district, Arunachal Pradesh	MH647526	Mahony et al. 2018
<i>Xenophrys himalayana</i>	SDBDU 2009.1206	India, West Kameng district, Arunachal Pradesh	MH647527	Mahony et al. 2018
<i>Xenophrys lancangica</i>	SYS a007794	China, Yunnan, Simao	OQ180994	Lyu et al. 2023
<i>Xenophrys lancangica</i>	SYS a007825	China, Yunnan, Jinghong	OQ180997	Lyu et al. 2023
<i>Xenophrys lekaguli</i>	FMNH 265955	Thailand, Sa Kao, Mueang Sa Kao	KY022214	Mahony et al. 2017
<i>Xenophrys major</i>	RGK 0089	India, Manipur, Tamenglong	KY022308	Mahony et al. 2017
<i>Xenophrys major</i>	SDBDU 2007.229	India, Nagaland, Kohima	MH647514	Mahony et al. 2018
<i>Xenophrys mangshanensis</i>	KIZ 021786	China, Guangdong, Mt Nanling	KX811790	Chen et al. 2017
<i>Xenophrys mangshanensis</i>	SYS a002177	China, Guangdong, Huaiji	MH406666	Liu et al. 2018
<i>Xenophrys maosonensis</i>	KIZ 016045	China, Yunnan, Xichou	KX811780	Chen et al. 2017
<i>Xenophrys maosonensis</i>	SYS a008748	China, Guangxi, Mt Shiwandashan	OQ181000	Lyu et al. 2023
<i>Xenophrys maosonensis</i>	SYS a008766	China, Guangxi, Mt Shiwandashan	OQ181002	Lyu et al. 2023
<i>Xenophrys medogensis</i>	KIZ 06657	China, Xizang, Beibeng	KX811768	Chen et al. 2017
<i>Xenophrys medogensis</i>	SYS a002932	China, Xizang, Medog	MH406725	Liu et al. 2018
<i>Xenophrys megacephala</i>	ZSI A 11213	India, Meghalaya, Ri Bhoi	KY022315	Mahony et al. 2018
<i>Xenophrys montana</i>	SDBDU 2011.1047	India, West Bengal, Darjeeling	KY022312	Mahony et al. 2017
<i>Xenophrys montana</i>	SDBDU 2011.1049	India, West Bengal, Darjeeling	MH647506	Mahony et al. 2018
<i>Xenophrys numhumaeng</i>	SDBDU 2007.041	India, Manipur, Tamenglong	KY022316	Mahony et al. 2017
<i>Xenophrys numhumaeng</i>	BNHS 6076	India, Manipur, Tamenglong	MN734393	Mahony et al. 2020
<i>Xenophrys oreocrypta</i>	SDBDU 2008.1400	India, West Garo Hills district, Meghalaya	MH647520	Mahony et al. 2018
<i>Xenophrys oreocrypta</i>	SDBDU 2009.1108	India, West Garo Hills district, Meghalaya	MH647521	Mahony et al. 2018
<i>Xenophrys oropedion</i>	ZSI A 11601	India, Meghalaya state, East Khasi Hills district, Shillong, Malki Forest	MN734394	Mahony et al. 2020
<i>Xenophrys oropedion</i>	ZSI A 11603	India, Meghalaya state, East Khasi Hills district, Shillong, Malki Forest	MN734395	Mahony et al. 2020
<i>Xenophrys pangdaensis</i>	YBU 21248	China, Xizang, Yadong County, Pangda Village	OR026569	Shu et al. 2023
<i>Xenophrys pangdaensis</i>	YBU 21261	China, Xizang, Yadong County, Pangda Village	OR026572	Shu et al. 2023
<i>Xenophrys periosa</i>	SDBDU 2009.793	India, East Siang district, Arunachal Pradesh	MH647522	Mahony et al. 2018
<i>Xenophrys periosa</i>	SDBDU 2009.1285	India, West Kameng district, Arunachal Pradesh	MH647524	Mahony et al. 2018
<i>Xenophrys periosa</i>	CIB YN201909160	China, Yunnan, Gongshan, Dulongjiang	MT225581	Shi et al. 2020

Species	Voucher	Locality	Accession No.	Reference
<i>Xenophrys periosa</i>	KIZ 053849	China, Yunnan, Tongbiguan	PP989324	Wu et al. 2024
<i>Xenophrys robusta</i>	K5207/ZSI11404	India, Sikkim, North Sikkim	KX894674	Deuti et al. 2017
<i>Xenophrys robusta</i>	SDBDU 2011.1057	India, West Bengal, Darjeeling	KY022314	Mahony et al. 2018
<i>Xenophrys serchhipii</i>	SDBDU 2009.612	India, Tripura, North Tripura	KY022323	Mahony et al. 2018
<i>Xenophrys serchhipii</i>	SDBDU 2008.1492	India, Manipur, Tamenglong	MN734405	Mahony et al. 2020
<i>Xenophrys yingjiangensis</i>	KIZ 011940	Myanmar, Myitkyina	KX811792	Chen et al. 2017
<i>Xenophrys yingjiangensis</i>	KIZ 048503	China, Yunnan, Tongbiguan Provincial Nature Reserve	KX811793	Chen et al. 2017
<i>Xenophrys yingjiangensis</i>	KIZ 048504	China, Yunnan, Tongbiguan Provincial Nature Reserve	KX811794	Chen et al. 2017
<i>Xenophrys yingjiangensis</i>	KIZ 048505	China, Yunnan, Tongbiguan Provincial Nature Reserve	KX811795	Chen et al. 2017
<i>Xenophrys yingjiangensis</i>	KIZ 053814	China, Yunnan, Tongbiguan, Nabang Town	PP989318	Wu et al. 2024
<i>Xenophrys yingjiangensis</i>	KIZ 053815	China, Yunnan, Tongbiguan, Nabang Town	PP989320	Wu et al. 2024
<i>Xenophrys yingjiangensis</i>	KIZ 053828	China, Yunnan, Tongbiguan Provincial Nature Reserve	PP989319	Wu et al. 2024
<i>Xenophrys yingjiangensis</i>	KIZ 053848	China, Yunnan, Tongbiguan Provincial Nature Reserve	PP989317	Wu et al. 2024
<i>Xenophrys</i> sp.2	KIZ 053846	China, Yunnan, Tongbiguan Provincial Nature Reserve	PP989321	Wu et al. 2024
<i>Xenophrys tongbiguanensis</i> sp. nov.	KIZ 058838	China, Yunnan, Tongbiguan Provincial Nature Reserve	PV820621	This study
<i>Xenophrys tongbiguanensis</i> sp. nov.	KIZ 058840	China, Yunnan, Tongbiguan Provincial Nature Reserve	PV820622	This study
<i>Xenophrys tongbiguanensis</i> sp. nov.	KIZ 058785	China, Yunnan, Tongbiguan Provincial Nature Reserve	PV820618	This study
<i>Xenophrys tongbiguanensis</i> sp. nov.	KIZ 058786	China, Yunnan, Tongbiguan Provincial Nature Reserve	PV820619	This study
<i>Xenophrys tongbiguanensis</i> sp. nov.	KIZ 058787	China, Yunnan, Tongbiguan Provincial Nature Reserve	PV820620	This study
<i>Xenophrys takensis</i>	FMNH 261711	Thailand, Kampaeng, Khlong Lan	KY022215	Mahony et al. 2017
<i>Xenophrys truongsonensis</i>	IEBRA 4943	Vietnam, Dak Lak	ON146200	Luong et al. 2022
<i>Xenophrys truongsonensis</i>	IEBRA 4948	Vietnam, Lam Dong	ON146201	Luong et al. 2022
<i>Xenophrys truongsonensis</i>	IEBRA 4952	Vietnam, Ninh Thuan	ON146202	Luong et al. 2022
<i>Xenophrys zhangii</i>	KIZ 014278	China, Xizang, Nyalam	KX811765	Chen et al. 2017
<i>Xenophrys zhangii</i>	SYS a008204	China, Xizang, Nyalam	OQ180998	Lyu et al. 2023
<i>Xenophrys zunhebotoensis</i>	RGK 0041	India, Nagaland, Zunheboto	KY022322	Mahony et al. 2017
<i>Xenophrys zunhebotoensis</i>	SDBDU 2009.374	India, Nagaland, Kohima	MN734418	Mahony et al. 2020
<i>Xenophrys apatani</i>	NE 3	India	MH423746	Saikia et al. 2019
Outgroups				
<i>Brachytarsophrys feae</i>	KIZ YN070570	China, Yunnan, Longchuan	KX811809	Chen et al. 2017
<i>Leptobrachella ventripunctata</i>	KIZ 046940	China, Yunnan, Wenlong	KX811929	Chen et al. 2017
<i>Leptobrachium huashen</i>	KIZ 049025	China, Yunnan, Mengyang	KX811931	Chen et al. 2017

Taxonomic account

Xenophrys tongbiguanensis Wu, Yu, Chen & Che, sp. nov.

<https://zoobank.org/2668BAF4-71E0-4F7B-A707-B22199992A7B>
Figs 3, 4, Table 3

Chresonymy. *Xenophrys* sp., Wu et al. 2024.

Type material. *Holotype* • KIZ 058786, an adult male collected from Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, CHINA (24.562476°N, 97.642761°E; elevation 1500 m a.s.l.), collected by

Shen-Pin Yang, Jia-Sheng Liu, and Shen-Quan Liu on 13 August 2024.

Paratypes • KIZ 058785 and KIZ 058786, two adult males, collected at the same locality with the same collection information as the holotype • KIZ 058838, an adult female, from Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, CHINA (97.675177°N, 24.781519°E; elevation 1452 m a.s.l.), collected by Shen-Pin Yang, Jia-Sheng Liu, and Shen-Quan Liu on 19 August 2024 • KIZ 058840, an adult female, from Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan,

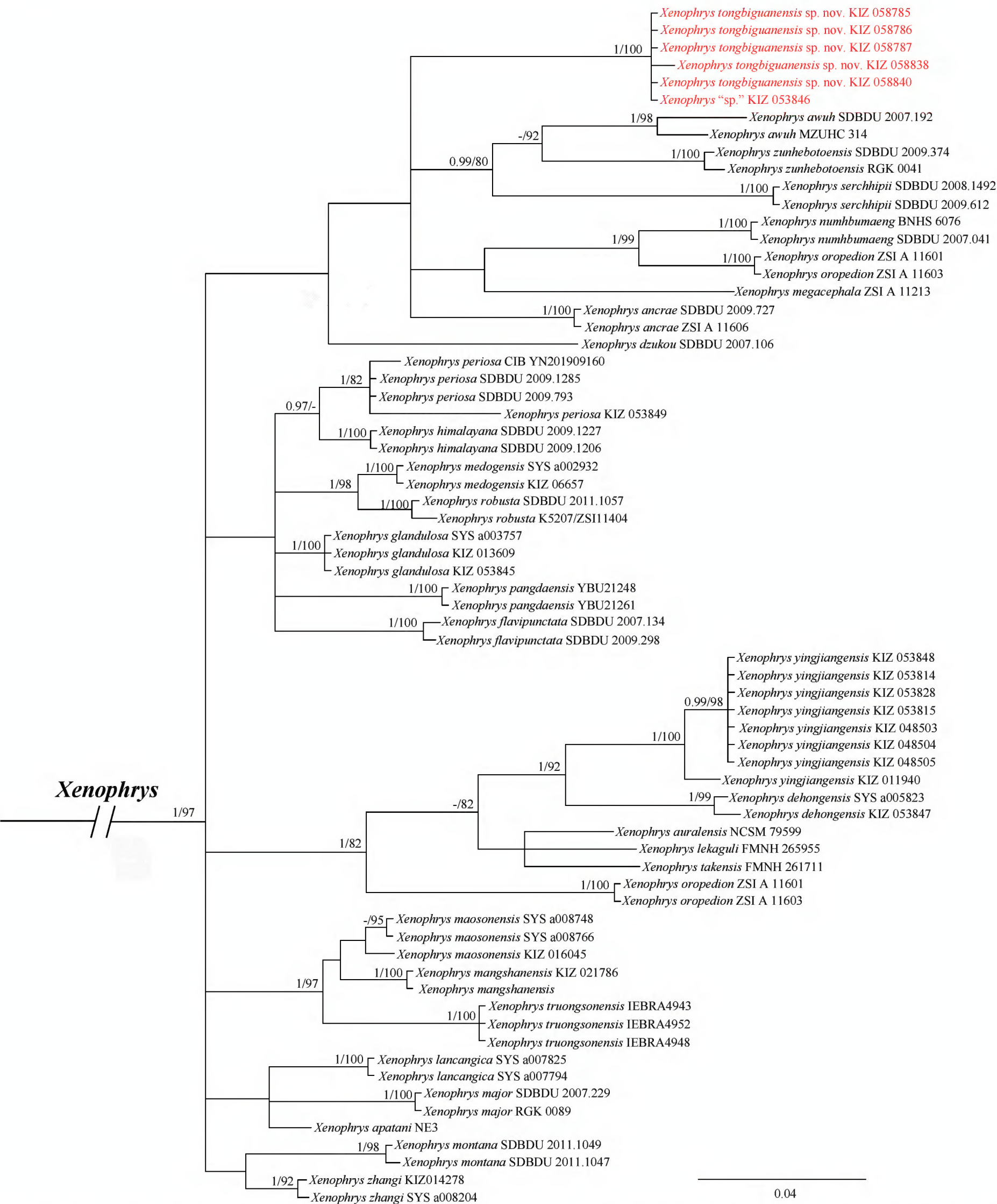


Figure 2. Phylogram of *Xenophrys* based on mitochondrial 16S rRNA gene. Node values with Bayesian posterior probabilities (BPP) < 0.95/bootstrapped support (BS) < 70 are not shown. A “–” denotes Bayesian posterior probabilities (BPP) < 0.95 or bootstrapped support (BS) < 70.

CHINA (97.830888°N, 25.124331°E; elevation 1563 m a.s.l.), collected by Shen-Pin Yang, Jia-Sheng Liu, and Shen-Quan Liu on 20 August 2024.

Etymology. The specific epithet “tongbiguanen” is a Latinized adjective derived from the name of Tongbiguan Provincial Nature Reserve, Yunnan Province, China, where

the new species occurs. We propose the English common name “Tongbiguanen horned toad” and the Chinese common name “Tóng Bì Guān Jiǎo Chán (铜壁关角蟾).”

Diagnosis. *Xenophrys tongbiguanensis* sp. nov. can be distinguished from its congeners by the following combination of morphological characters: (1) body size medium,

Table 2. Average uncorrected p-distances (percentage) among *Xenophrys* species calculated from 16S rRNA gene sequences (below the diagonal) and standard error estimates (above the diagonal). The ingroup mean uncorrected p-distances are shown on the diagonal.

Species name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>Xenophrys tongbiguanensis</i> sp. nov.	0.4	3.6	3.4	3.8	3.8	3.5	3.6	3.8	4.0	3.4	3.5	3.7	4.9	3.9	4.2	3.7	3.8	4.5	3.2	4.2	3.7	3.6	3.6	3.5	4.1	3.9	4.5	3.6	3.3	4.6
<i>Xenophrys apatani</i>	10.9	–	2.8	3.7	1.9	1.7	3.2	4.1	3.5	2.9	2.8	2.9	4.4	3.0	4.0	2.4	3.2	3.8	2.5	3.8	2.5	2.2	2.2	2.4	3.0	3.7	3.8	2.6	3.4	4.3
<i>Xenophrys dzukou</i>	9.6	6.7	–	3.6	2.8	2.9	2.8	4.0	3.5	1.9	3.0	3.5	4.4	3.7	3.7	3.1	3.5	3.8	2.9	3.0	3.3	3.3	3.3	3.1	3.3	3.9	3.9	3.3	3.6	4.6
<i>Xenophrys dehongensis</i>	12.9	11.3	10.7	2.7	4.1	3.3	3.4	4.2	4.2	3.7	3.5	4.1	3.8	3.8	4.3	3.8	4.0	2.9	3.6	3.1	3.7	3.5	3.5	3.8	3.9	4.1	3.8	3.9	4.3	3.0
<i>Xenophrys pangdaensis</i>	12.2	2.7	6.7	14.0	0.0	2.5	3.2	4.3	3.8	2.8	2.9	2.6	4.3	2.6	3.9	2.9	3.4	3.3	2.9	3.3	3.2	2.6	2.4	2.8	3.0	3.8	3.5	2.9	3.6	4.2
<i>Xenophrys lancangica</i>	10.9	2.7	6.7	8.7	5.3	0.0	3.1	4.0	3.4	2.9	2.6	2.9	4.2	2.9	4.0	2.5	3.1	3.7	1.9	3.6	2.8	2.1	2.1	2.4	3.0	3.7	4.0	2.6	3.8	4.2
<i>Xenophrys truongsoneensis</i>	12.2	8.0	6.7	10.0	8.0	8.0	0.0	4.1	4.0	3.1	3.1	3.5	3.8	3.1	3.8	3.6	3.4	3.3	3.1	2.7	3.4	2.6	2.7	3.0	3.3	3.6	3.7	3.3	3.8	4.3
<i>Xenophrys awuh</i>	14.4	16.0	16.0	17.3	17.3	14.7	16.0	2.7	3.8	3.7	4.1	3.9	5.1	4.1	4.3	3.9	3.9	4.4	3.8	4.3	3.9	4.0	4.0	3.9	4.1	3.1	4.6	4.0	2.6	4.9
<i>Xenophrys numhumaeng</i>	14.9	10.7	10.7	14.7	13.3	10.7	14.7	14.7	0.0	2.9	3.5	4.0	5.1	4.1	3.2	3.0	4.0	4.6	3.6	4.2	3.5	3.9	3.9	3.8	4.1	3.6	4.4	3.9	3.6	4.8
<i>Xenophrys ancræe</i>	9.6	6.7	2.7	10.7	6.7	6.7	8.0	13.3	8.0	0.0	2.9	3.4	4.6	3.5	3.2	2.5	3.5	3.9	2.9	3.5	3.3	3.3	3.3	3.2	3.3	3.4	3.8	3.3	3.2	4.7
<i>Xenophrys flavipunctata</i>	11.6	7.3	7.3	10.7	7.3	6.0	7.3	16.0	11.3	7.3	1.3	3.1	4.0	3.2	3.6	3.5	3.2	3.7	2.6	3.5	2.5	2.8	2.8	2.7	3.0	3.5	4.1	2.9	3.8	4.4
<i>Xenophrys himalayana</i>	13.1	6.7	10.7	14.0	5.3	6.7	9.3	13.3	16.0	10.7	8.7	0.0	4.1	1.5	4.3	3.4	2.6	3.6	2.1	3.6	3.4	2.7	2.5	2.0	2.4	3.3	3.6	2.1	3.6	4.0
<i>Xenophrys oreocrypta</i>	21.6	16.0	14.7	12.7	14.7	13.3	12.0	24.0	20.0	16.0	12.7	14.7	0.0	3.8	4.4	4.8	4.6	3.5	3.9	3.8	4.8	4.0	4.0	4.0	3.9	4.9	4.2	4.1	5.1	4.0
<i>Xenophrys periosa</i>	15.1	7.7	12.0	12.7	6.3	7.7	8.0	15.3	17.3	12.0	10.0	2.7	13.7	2.4	4.0	3.5	3.0	3.2	2.6	3.2	3.6	2.3	2.0	2.5	2.6	3.4	3.3	2.7	3.9	3.8
<i>Xenophrys oropedion</i>	16.2	13.3	10.7	14.7	13.3	13.3	12.0	18.7	8.0	8.0	11.3	17.3	14.7	16.0	0.0	3.6	4.3	4.2	4.0	3.8	4.2	3.9	3.9	4.2	4.2	3.8	4.0	4.2	3.9	4.8
<i>Xenophrys megacephala</i>	12.2	5.3	8.0	12.0	8.0	5.3	12.0	14.7	8.0	5.3	11.3	12.0	18.7	13.0	10.7	–	3.7	4.0	3.0	3.9	3.4	3.1	3.1	3.3	3.6	3.5	3.8	3.4	3.4	4.4
<i>Xenophrys medogensis</i>	13.1	8.0	10.7	14.0	9.3	8.0	9.3	13.3	16.0	10.7	8.7	5.3	18.7	7.7	17.3	13.3	0.0	4.2	2.9	3.9	3.3	3.0	3.0	2.7	2.4	3.5	4.1	2.9	3.8	4.7
<i>Xenophrys auralensis</i>	17.6	10.7	12.0	8.0	8.0	10.7	8.0	17.3	17.3	12.0	11.3	9.3	10.7	8.0	14.7	13.3	13.3	–	3.7	2.3	4.2	3.4	3.2	3.6	3.6	4.1	2.8	3.8	4.3	3.0
<i>Xenophrys glandulosa</i>	9.6	5.3	6.7	10.0	6.7	2.7	8.0	13.3	12.0	6.7	6.0	4.0	12.0	6.7	13.3	8.0	6.7	10.7	0.0	3.6	3.1	2.2	2.2	1.4	2.7	3.6	4.0	1.7	3.5	4.1
<i>Xenophrys lekaguli</i>	16.2	10.7	8.0	9.3	8.0	10.7	5.3	17.3	16.0	10.7	10.0	9.3	12.0	8.0	13.3	13.3	12.0	4.0	10.7	–	3.9	3.3	3.1	3.6	3.8	3.7	3.0	3.8	4.0	3.5
<i>Xenophrys major</i>	12.2	5.3	9.3	11.3	8.0	6.7	9.3	14.7	10.7	9.3	6.0	9.3	18.7	10.7	16.0	10.7	9.3	13.3	8.0	12.0	0.0	3.1	3.2	2.9	3.3	3.7	4.4	3.1	3.8	4.5
<i>Xenophrys mangshanensis</i>	12.2	4.0	9.3	10.0	5.3	4.0	5.3	14.7	14.7	9.3	7.3	5.3	13.3	4.7	13.3	9.3	6.7	8.0	4.0	8.0	8.0	0.0	0.4	2.0	2.9	3.4	3.8	2.3	3.5	4.1
<i>Xenophrys maosonensis</i>	12.7	4.4	9.8	10.4	4.9	4.4	5.8	15.1	15.1	9.8	7.8	4.9	13.3	4.2	13.8	9.8	7.1	7.6	4.4	7.6	8.4	0.4	0.9	2.0	3.0	3.4	3.6	2.4	3.5	4.0
<i>Xenophrys montana</i>	11.6	4.7	8.7	12.0	6.0	4.7	7.3	14.0	14.0	8.7	6.7	3.3	14.0	6.0	15.3	10.0	6.0	10.0	2.0	10.0	7.3	3.3	3.8	0.3	2.5	3.4	4.0	1.4	3.4	4.1
<i>Xenophrys robusta</i>	15.1	7.3	10.0	13.3	7.3	7.3	8.7	14.7	16.7	10.0	8.0	4.7	14.0	6.3	16.7	12.7	4.7	10.0	6.0	11.3	10.0	7.3	7.8	5.3	0.3	3.7	3.8	2.7	4.0	4.2
<i>Xenophrys serchhipii</i>	14.4	12.0	13.3	14.7	13.3	12.0	10.7	9.3	13.3	10.7	11.3	9.3	20.0	10.3	13.3	12.0	9.3	13.3	10.7	12.0	13.3	9.3	9.8	10.0	11.3	0.0	4.0	3.6	3.0	4.5
<i>Xenophrys takensis</i>	18.4	12.0	13.3	14.7	9.3	14.7	12.0	18.7	18.7	13.3	15.3	10.7	17.3	9.3	16.0	13.3	14.7	6.7	14.7	8.0	16.0	12.0	11.6	14.0	12.7	14.7	–	4.1	4.2	3.7
<i>Xenophrys zhangji</i>	12.2	5.3	9.3	12.7	6.7	5.3	8.0	14.7	14.7	9.3	7.3	4.0	14.7	6.7	16.0	10.7	6.7	10.7	2.7	10.7	8.0	4.0	4.4	2.0	6.0	10.7	14.7	0.0	3.6	4.2
<i>Xenophrys zunhebotoensis</i>	9.6	9.3	10.7	16.0	10.7	12.0	12.0	6.7	12.0	8.0	12.7	12.0	22.7	14.0	13.3	9.3	12.0	14.7	10.7	13.3	12.0	10.7	11.1	10.0	14.0	8.0	14.7	10.7	0.0	4.8
<i>Xenophrys yingjiangensis</i>	20.2	14.7	18.7	9.3	14.7	14.7	14.7	22.7	21.3	18.7	16.7	13.3	16.0	12.3	21.3	16.2	18.7	8.0	14.7	10.7	16.0	13.3	12.9	14.0	15.3	18.7	12.3	14.7	20.0	0.7

SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females; (2) head length almost equal to width; (3) vomerine teeth prominent; (4) eye diameter less than twice as long as maximum tympanum diameter; (5) tympanum distinct, upper margin concealed by supratympanic fold; (6) tongue oval-shaped, slightly notched posteriorly; (7) tibio-tarsal articulation of straightened limb reaching between nostrils and tip of snout; (8) the heels slightly overlapping; (9) relative finger length: $I < II < IV < III$; (10) metacarpal tubercle absent; (11) toes with rudimentary webbing; (12) throat, chest, and anterior half of abdomen yellowish-brown with dense orange flecking and three longitudinal dark-brown stripes that one located in the middle of the throat and the other two located below the pectoral glands; (12) male with single internal vocal sac; and (13) nuptial pads in males absent.

Description of holotype. (measurements in Table 3): KIZ 058786, mature male, sized medium body (SVL 44.2 mm); head slightly longer than wide (HL/HW = 1.02); snout bluntly pointed in dorsal view, obtusely protruding in lateral view, notably projecting beyond lower jaw; triangular in dorsal view; top of head flat; loreal region vertical and concave; canthus rostralis distinct, sharp (Fig. 3A); pupil diamond-shaped, vertical (Fig. 3B); eyes large (ED/HDL 29.0%), eye diameter less than twice as long as maximum tympanum diameter (TD/ED 52.9%) and shorter than snout length (SNT 6.1 mm, ED/SNT 83.6%); nares oval, laterally orientated, located much closer to eye than tip of snout (SN 3.4 mm, DNE 2.7 mm); eye-tympanum distance larger than maximum tympanum diameter (TEY/TD 114.9%); tympanum distinct, oval-shaped, relatively small (TD/HDL 15.3%), with upper ~10% concealed by supratympanic ridge; internarial distance almost equal to interorbital distance (IND/IOD 101.8%), and larger than width of upper eyelid (IND/UEW 131.8%); tongue oval-shaped, slightly notched posteriorly; maxillary teeth present; vomerine teeth prominent, in two oblique series, positioned between choanae; pineal ocellus absent; single internal vocal sac, with a sac slit opening on floor of mouth at each corner (Fig. 3A, B).

Forelimbs moderately long and slender (HL/SVL 29.0%; FHL/SVL 53.6%); forearm not enlarged, its length shorter than the hand length (FAL/HL 70.3%); fingers long and narrow, not flattened dorsoventrally; lateral fringes on fingers absent; finger length from shortest to longest: $I < II < IV < III$; tips of all fingers rounded, slightly dilated relative to digit widths, with circular pads, terminal grooves absent; no webbing between fingers; subarticular tubercle absent; supernumerary tubercle absent; inner metacarpal tubercle and outer metacarpal tubercle indistinct; nuptial pads absent (Fig. 3E).

Hindlimbs long and robust, thigh length shorter than the tibia length (THL/TL 96.6%) but greater than the foot length (THL/FL 108.5%); tibia length 53.6% of SVL; foot length 47.7% of SVL; tibio-tarsal articulation of straightened limb reaching between nostrils and tip of snout when adpressed along body; the heels slightly overlapping when the tibias are perpendicular to the body axis;

toes long and slightly dorsoventrally flattened, toe length from shortest to longest: $I < II < V < III < IV$; tips of all toes rounded, slightly dilated, terminal grooves absent; notably expanded relative to digit widths, forming circular pads; terminal grooves absent; lateral dermal fringes on toes weak, narrow; rudimentary webbing present between all toes; subarticular tubercle absent, replaced by indistinct callous dermal ridges; supernumerary tubercle absent; inner metatarsal tubercle indistinct, oval-shaped, outer metatarsal tubercle absent (Fig. 3F).

Skin of dorsal surfaces of body, limbs, and dorsal and lateral surfaces of head primarily smooth, with scattered weakly granular; flanks with several large distinct warts (Fig. 3A); a small pointed horn-like tubercle present on the outer edge of the upper eyelid (Fig. 3B); supratympanic fold distinct, narrow anteriorly, slightly widening posteriorly, curves down broadly through upper tympanum, terminating above forelimbs; dorsolateral fold absent; a distinct narrow ‘\’-shaped parietoscapular ridge present, its two sides extending posteriorly from above tympanum, terminating beyond level of axilla (Fig. 3A); dorsal surface with distinct and irregular transverse ridges; ventral surface smooth; pectoral glands small, rounded, slightly raised, positioned on level with axilla; femoral gland distinct, located on the rear of each thigh at midpoint between knee and cloacal opening; pectoral glands slightly larger than femoral gland (Fig. 3D, F).

Coloration in life. Dorsum reddish brown, with a complete dark brown inverted triangle marking between the eyes, border of marking with a light edge; distinct dark brown X-shaped marking on dorsum; three to four dark brown vertical bars present on upper lips, the one below eye largest; lower lip black with four white bars, symmetrically distributed; canthus rostralis, supratympanic fold, to upper eyelid with a light edge; temporal region under supratympanic ridge dark brown, with a light edge; tubercles dark brown with cream-white tips; dorsal surface of forelimbs, hindlimbs, and fingers with dark crossbars; throat, chest, and anterior half of abdomen yellowish-brown with dense orange flecking and three longitudinal dark-brown stripes that one located in the middle of the throat and the other two located below the pectoral glands; posterior half of abdomen white with scattered orange flecking; ventral surface of thighs white, scattered small grey blotches; ventral surface of feet and shanks brown-black; pectoral and femoral glands cream-white; iris copper-brown with tiny radiating dark lines; pupil black, outlined in yellowish-brown; metatarsal tubercle, tips of finger, and toe orange-red.

Coloration in preservative. After seven months of preservation in ethanol, the dorsal surface of head, back, and limbs fading to greyish-brown; a solid darker brown triangular marking between eyes; dark brown crossbars on dorsal surface of forelimbs, hindlimbs, and fingers still clear; throat, chest, and abdomen faded to pale grey-white, with greyish-brown flecking; three longitudinal stripes of the venter still clear, dark brown; metatarsal tubercle, tips of fingers and toes faded greyish-brown; pectoral and femoral glands white; toe webbing dark brown (Fig. 4).



Figure 3. Views of the holotype KIZ 058786 in life. **A.** Lateral view; **B.** Lateral view of head; **C.** Dorsal view; **D.** Ventral view; **E.** Ventral view of hand, and **F.** Ventral view of foot. Photos by Zhong-Bin Yu.

Sexual dimorphism. Adult female specimens have larger body sizes than adult male specimens (SVL 54.8–57.6 mm vs. 43.5–44.2 mm, Table 3). Male with a single internal vocal sac, with a sac slit opening on floor of the mouth at each corner.

Distribution and ecology. Currently, *Xenophrys tongbiguanensis* sp. nov. is known only from the type locality, Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan Province, China (Fig. 1). This frog inhabits flowing montane streams and the nearby forest floor in subtropical evergreen broad-leaved forests (Fig. 5). The frog usually perches on cardamom leaves at night, but it can be found among piles of dead leaves by the roadside on rainy days. Advertisement calls of males were heard in August. However, the breeding season is currently un-

clear. The sympatric species *Leptobranchella ventripunctata*, *Zhangixalus smaragdinus*, *Rhacophorus rhodopus*, and *Limnonectes longchuanensis* were also recorded.

Comparison. We compared *Xenophrys tongbiguanensis* sp. nov. with other congeneric species (Ohler et al. 2002; Stuart et al. 2006; Mahony 2011; Mahony et al. 2011, 2013, 2018, 2020; Che et al. 2020; Luong et al. 2022; Lyu et al. 2023; Shu et al. 2023; Saikia et al. 2024; Wu et al. 2024).

Xenophrys tongbiguanensis sp. nov. is clearly distinct from its four sympatrically distributed congeners (*X. periosa*, *X. dehongensis*, *X. glandulosa*, and *X. yingjiangensis*). It differs from *X. periosa* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 71.3–93.8 mm in 12 adult males and

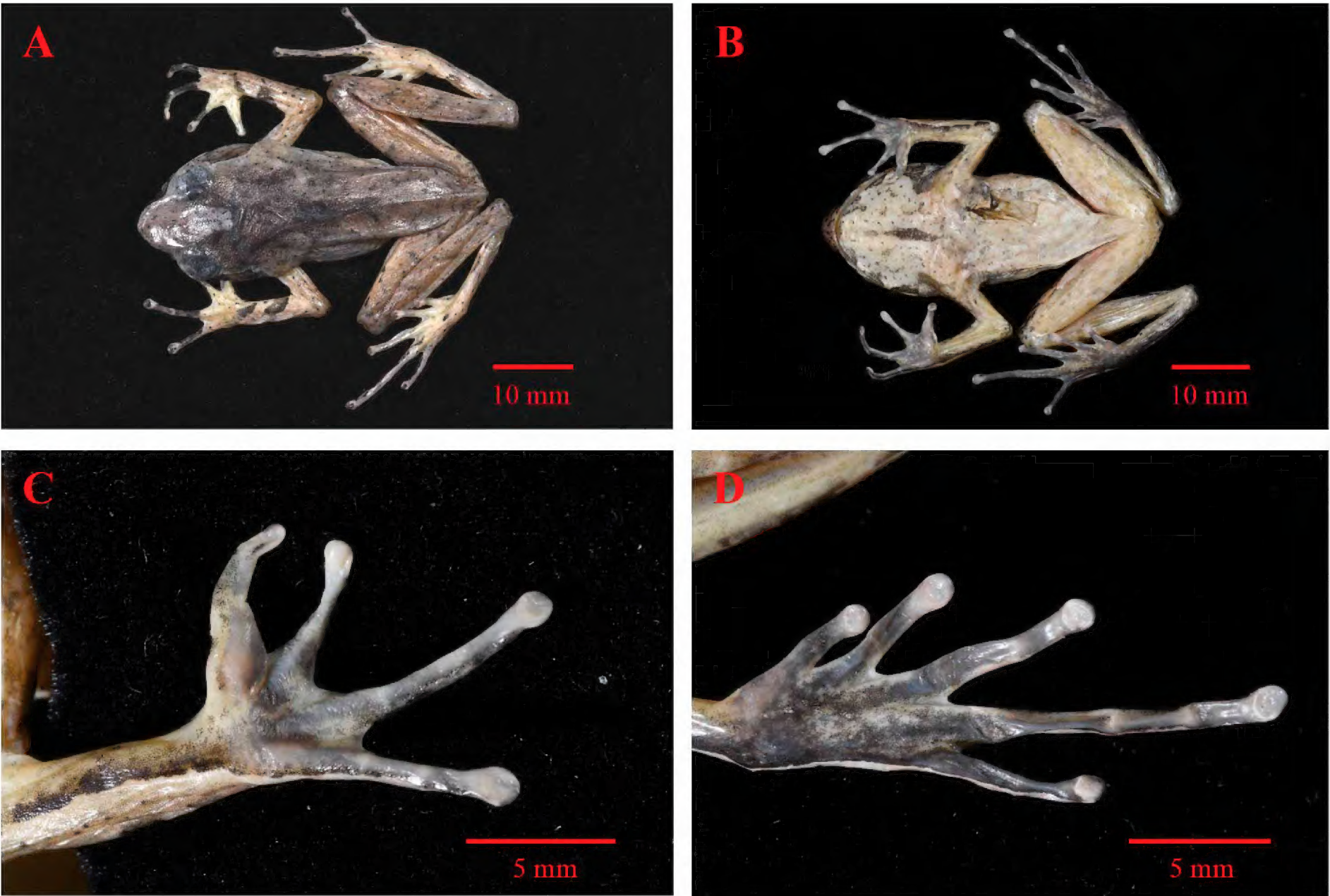


Figure 4. Views of the holotype KIZ 058786 in preservative. **A.** Dorsal view; **B.** Ventral view; **C.** Ventral view of hand, and **D.** ventral view of foot. Photos by Zhong-Bin Yu.

Table 3. Measurements (in mm) of the type series of *Xenophrys tongbiguanensis* sp. nov. An asterisk (*) indicates the holotype.

	KIZ 058838	KIZ 058840	KIZ 058787	KIZ 058786*	KIZ 058785
Sex	♀	♀	♂	♂	♂
SVL	57.6	54.8	43.5	44.2	43.8
HDL	21.7	20.6	16.9	17.6	16.8
HDW	22.7	20.5	17.3	17.2	16.4
SNT	8.1	7.6	6.4	6.1	6.0
ED	6.1	6.0	5.6	5.1	4.8
IOD	6.5	6.4	5.6	5.7	5.4
UEW	5.8	5.0	4.5	4.4	4.6
IND	7.2	7.0	6.0	5.8	5.7
DNE	3.8	3.2	2.8	2.7	2.7
SN	4.0	4.0	3.3	3.4	3.2
TD	3.7	3.7	2.7	2.7	2.8
TEY	4.2	4.0	3.4	3.1	3.2
FHL	30.2	27.0	23.1	23.7	22.8
HL	17.7	15.1	12.7	12.8	12.6
LAD	5.2	4.0	3.7	3.5	3.3
FAL	10.9	10.3	9.1	9.0	8.8
HLL	98.0	89.1	75.5	78.2	72.9
THL	28.0	25.7	22.6	22.9	21.2
TL	29.8	27.5	23.1	23.7	22.6
TAL	43.4	39.1	32.9	33.5	32.0
FL	28.2	24.1	19.8	21.1	20.3

112.0 mm in one adult female), outer metacarpal tubercle absent (vs. outer metacarpal tubercle weakly developed), iris copper-brown (vs. iris very dark orange), and nuptial pads in males absent (vs. present); from *X. dehongensis* by a larger body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 34.8–

36.7 mm in five adult males and 45.7–46.8 mm in two adult females), nuptial spines in males absent (vs. dense black nuptial spines on dorsal bases of fingers I and II), and tibio-tarsal articulation of straightened limb reaching between nostrils and tip of snout (vs. reaching posterior corner of eye); from *X. glandulosa* by a smaller body size,



Figure 5. Habitat at the type locality of *Xenophrys tongbiguanensis* sp. nov. at Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, China. (Photo by Zhong-Bin Yu).

43.5–44.2 mm in three adult males (vs. SVL 77.0–81.0 mm in three adult males), nuptial pads in males absent (vs. present), and lateral dermal fringes on toes distinct, narrow (vs. moderately wide lateral fringes present on all toes); from *X. yingjiangensis* by inner metatarsal tubercle indistinct, oval-shaped (vs. inner metatarsal tubercle large, elongate), relative finger lengths: I < II < IV < III (vs. II < IV < I < III), nuptial pads in males absent (vs. present), dorsal surface of limbs with dark crossbars (vs. absent), and three to four dark brown vertical bars below eye (vs. absent).

Xenophrys tongbiguanensis sp. nov. is different from other congeneric species. The new species differs from *X. ancrae* by pupil diamond-shaped, vertical (vs. pupil oval, horizontally orientated), iris copper-brown (vs. iris metallic mid-brown), and nuptial spines in males absent (vs. present); from *X. apatani* by tongue oval-shaped, slightly notched posteriorly (vs. tongue entire and rounded), relative finger length: I < II < IV < III (vs. I < II = IV < III), a smaller body size, 43.5–44.2 mm in three adult males (vs. SVL 54.0 mm in one adult male), eye diameter less than twice as long as maximum tympanum diameter (vs. eye diameter twice the diameter of tympanum), and head length almost equal to wide (vs. head moderately large, wider than long); from *X. auralensis* by a smaller body size, 43.5–44.2 mm in three adult males (vs. SVL 71.0–76.9 mm in nine adult males), tongue oval-shaped, slightly notched posteriorly (vs. tongue entire and oval), vomerine teeth prominent (vs. absent), and inner metatarsal tubercle indistinct, oval-shaped (vs. distinct and long); from *X. awuh* by vomerine teeth prominent (vs. absent), lateral dermal fringes on toes distinct, narrow (vs. absent), metacarpal tubercle absent (vs. thenar and outer metacarpal tubercles weak), nuptial pads in males absent (vs. present), and tympanum distinct, upper margin concealed by supratympanic fold (vs. tympanum distinctly oval shaped, upper border not concealed by supratympanic ridge); from *X. damrei* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 57.1 mm in one adult male and 69.1 mm in one adult female), nuptial pads in males absent (vs. present), head length almost equal to wide (vs. head considerably wider than long), and male with single internal

vocal sac (vs. external vocal sac indistinct); from *X. dzukou* by a larger body size, SVL 43.5–44.2 mm in three adult males (vs. 34.2–35.3 mm in four adult males), eye diameter less than twice as long as maximum tympanum diameter (vs. eye length more than twice maximum tympanum diameter), tympanum distinct, upper margin concealed by supratympanic fold (vs. tympanum circular, upper border not concealed by supratympanic ridge), and toes with rudimentary webbing (vs. webbing absent); from *X. flavipunctata* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 56.9–68.4 mm in four adult males and 68.0–74.6 mm in three adult females), tongue oval-shaped, slightly notched posteriorly (vs. tongue moderately large, deeply notched posteriorly), and eye diameter less than twice as long as maximum tympanum diameter (vs. eye diameter 2.5 times maximum diameter of visible portion of tympanum); from *X. himalayana* by a smaller body size, SVL 43.5–44.2 mm in three adult males (vs. SVL 68.0–73.5 mm in six adult males), eye diameter less than twice as long as maximum tympanum diameter (vs. eye diameter more than twice maximum diameter of visible portion of tympanum), and relative finger length: I < II < IV < III (vs. IV < II < I < III); from *X. lancangica* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 64.0–65.4 mm in three adult males and 75.0–88.6 mm in three adult females), relative finger length: I < II < IV < III (vs. II < IV < I < III), and nuptial pads in males absent (vs. brown nuptial pads on dorsal bases of fingers I and II in adult males); from *X. lekaguli* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 56.6–66.6 mm in eight adult males and 71.8–94.0 mm in four adult females), relative finger length: I < II < IV < III (vs. IV < II < I < III), and tongue oval-shaped, slightly notched posteriorly (vs. tongue unnotched); from *X. major* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 75.0–87.5 mm in 12 adult males and 85.6–98.2 mm in three adult females), nuptial pads in males absent (vs. present), male with single internal vocal sac (vs. external vocal sac indistinct), and eye diameter less than twice as long as maximum tympanum diameter (vs. eye diameter 2.5 times maximum diameter of visible portion of tympanum); from *X. mangshanensis* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 60.4–71.6 mm in ten adult males and 62.0–77.9 mm in three adult females), relative finger length: I < II < IV < III (vs. II < I < IV < III), tibio-tarsal articulation of straightened limb reaching between nostrils and tip of snout (vs. reaching center of eye), toes with rudimentary webbing (vs. absent), and nuptial spines in males absent (vs. dense brown nuptial spines on dorsal bases of fingers I and II in adult males); from *X. maosonensis* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 66.2 mm in one adult male and 76.9 mm in one adult female), nuptial pads in males absent (vs. black nuptial pads on dorsal bases of fingers I and II in adult male),

and tibio-tarsal articulation of straightened limb reaching between nostrils and tip of snout (vs. reaching center of eye); from *X. medogensis* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 56.1–62.4 mm in six adult males and 73.8–79.6 mm in one adult females), relative finger length: $I < II < IV < III$ (vs. $II < I < IV < III$), and nuptial spines in males absent (vs. dense black nuptial spines on dorsal bases of fingers I and II in adult males); from *X. megacephala* by the heels slightly overlapping when the tibias positioned at right angles to the body axis (vs. not meeting), relative finger length: $I < II < IV < III$ (vs. $IV < II < I < III$), tongue oval-shaped, slightly notched posteriorly (vs. tongue long and narrow, without notch posteriorly), and nuptial pads in males absent (vs. present); from *X. monticola* by nuptial spines in males absent (vs. present), male with single internal vocal sac (vs. large subgular external vocal sac distinct), eye diameter less than twice as long as maximum tympanum diameter (vs. eye diameter more than twice as long as maximum diameter of visible portion of tympanum), and pupil vertically elliptical (vs. pupil horizontally orientated); from *X. numhumaeng* by a larger body size, SVL 43.5–44.2 mm in three adult males (vs. 33.8–34.6 mm in two adult males), pupil vertically elliptical (vs. pupil horizontally orientated), eye diameter less than twice as long as maximum tympanum diameter (vs. eye length more than two times longer than maximum tympanum diameter), single internal vocal sac (vs. external vocal sac indistinct), and nuptial pads in males absent (vs. present); from *X. oreocrypta* by a smaller body size, 54.8–57.6 mm in two adult females (vs. 94.9 mm in one adult female), throat, chest, and anterior half of abdomen yellowish-brown with dense orange flecking and three longitudinal dark-brown stripes, posterior half of abdomen white with scattered orange flecking (vs. ventral surface of throat and chest primarily pale grey with dark orange speckling, speckling more dense on lateral stripes of throat, and ventrolaterally on anterior abdomen), and relative finger length: $I < II < IV < III$ (vs. $IV < II < I < III$); from *X. oropedion* by a larger body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 32.8–39.2 mm in seven adult males and 44.1–48.7 mm in two adult females), and tympanum distinct, upper margin concealed by supratympanic fold (vs. tympanum slightly oval, vertically orientated, not concealed by supratympanic ridge); from *X. pangdaensis* by a larger body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 17.9–22.2 mm in six adult males and 23.4 mm in one adult female), and single internal vocal sac (vs. external vocal sac indistinct); from *X. parva* by relative finger length: $I < II < IV < III$ (vs. $II < IV < I < III$), tibio-tarsal articulation of straightened limb reaching between nostrils and tip of snout (vs. tibiotarsal articulation reaching anterior corner of eye), nuptial spines in males absent (vs. dense black nuptial spines on dorsal bases of fingers I and II in adult males), and throat, chest, and anterior half of abdomen yellowish-brown with dense orange flecking (vs. throat and chest purplish); from *X. robusta* by a smaller body size, SVL

43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 73.5–83.1 mm in six adult males and 81.3–108.3 mm in four adult females), nuptial pads in males absent (vs. present), single internal vocal sac (vs. external vocal sac indistinct), and vomerine teeth prominent (vs. absent); from *X. serchhipii* by relative finger length: $I < II < IV < III$ (vs. $IV < I = II < III$), nuptial pads in males absent (vs. present), and single internal vocal sac (vs. external vocal sac distinct); from *X. takensis* by head length almost equal to wide (vs. head wider than long), tongue oval-shaped, slightly notched posteriorly (vs. tongue oval, not notched posteriorly), nuptial pads in males absent (vs. present), relative finger lengths: $I < II < IV < III$ (vs. $IV \leq II < I < III$ or $IV = I < II < III$) and single internal vocal sac (vs. external vocal sac indistinct); from *X. truongsongensis* by a smaller body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 58.8–71.4 mm in 20 adult males and 65.6–87.3 mm in five adult females), and nostril closer to eye than tip of snout (vs. nostril closer to the tip of snout than to the eye); from *X. zhangii* by rudimentary webbing present between all toes (vs. toes without webbing), tibio-tarsal articulation of straightened limb reaching between nostrils and tip of snout (vs. reaching anterior corner of eye), and nuptial spines in males absent (vs. dense grey nuptial spines on dorsal bases of fingers I and II in adult males); from *X. zunhebotoensis* by a larger body size, SVL 43.5–44.2 mm in three adult males and 54.8–57.6 mm in two adult females (vs. 28.4–33.9 mm in 22 adult males and 37.0–39.5 mm in three adult females), vomerine teeth prominent (vs. absent), tympanum distinct, upper margin concealed by supratympanic fold (vs. tympanum circular, not concealed by supratympanic ridge), single internal vocal sac (vs. external vocal sac distinct), and nuptial spines in males absent (vs. present).

Discussion

The Tongbiguan Provincial Nature Reserve may harbor more hidden herpetological diversity than previously postulated. It is one of the richest regions in Yunnan for amphibians and reptiles. In recent years, a series of new species, new record genera, new national record species, and new regional record species have been discovered in the Tongbiguan Provincial Nature Reserve (e.g., Yang et al. 2018; Yang and Chan 2018; Liu et al. 2024; Wu et al. 2024, 2025; Xu et al. 2024; Yu et al. 2024; Wang et al. 2024). In addition, some studies have shown that cryptic species of amphibians and reptiles exist in this region (e.g., Chen et al. 2017, 2018; Wu et al. 2023). Our new finding of *X. tongbiguanensis* sp. nov. further confirms that the herpetological biodiversity of Tongbiguan Provincial Nature Reserve is yet to be fully documented.

Considering that Tongbiguan Provincial Nature Reserve is located on the China–Myanmar border, these regions share a similar zoogeographic setting. Recent studies have also confirmed that many species are co-distributed in China and Myanmar (e.g., Rahman et al. 2020; Wang

et al. 2024; Yu et al. 2024). Therefore, it is likely that this new species might also be present in northern Myanmar. As a result, we recommend international collaboration to strengthen fieldwork along the China–Myanmar border in order to further clarify the full range of this species.

Our study further reveals that species diversity within the genus *Xenophrys* remains insufficiently explored. Recent phylogenetic analyses have revealed multiple genetic lineages of *Xenophrys* that may represent new species (Chen et al. 2017; Wu et al. 2024). Among these, *X. tongbiguanensis* sp. nov. was previously suggested to be a putative new species based on molecular phylogenetic analysis (Wu et al. 2024). However, Wu et al. (2024) only included one subadult specimen and therefore could not describe it. During the field survey in August 2024, we collected five adult specimens of this new species (three adult males and two adult females). We combined morphological and molecular lines of evidence to confirm its species status and formally describe it. The discovery of *X. tongbiguanensis* sp. nov. brings the total number of *Xenophrys* species to 32 and the number known from China to 13. In addition, we recorded five species of *Xenophrys* from Tongbiguan Provincial Nature Reserve (Wu et al. 2024; Frost 2025): *X. dehongensis*, *X. glandulosa*, *X. periosa*, *X. yingjiangensis*, and *X. tongbiguanensis* sp. nov.

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References

Che J, Jiang K, Yan F, Zhang YP (2020) Amphibians and Reptiles of Tibet – Diversity and Evolution. Science Press, Beijing. [In Chinese]
 Chen JM, Zhou WW, Poyarkov Jr NA, Stuart BL, Brown RM, Lathrop A, Wang YY, Yuan ZY, Jiang K, Hou M, Chen HM, Suwannapoom C, Nguyen SN, Duong TV, Papenfuss TJ, Murphy RW, Zhang YP, Che J (2017) A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asian horned toads, genus *Megophrys* sensu lato (Anura: Megophryidae). *Molecular Phylogenetics and Evolution* 106: 28–43. <https://doi.org/10.1016/j.ympev.2016.09.004>
 Chen JM, Poyarkov NA, Suwannapoom C, Lathrop A, Wu YH, Zhou WW, Yuan ZY, Jin JQ, Chen HM, Liu HQ, Nguyen TQ, Nguyen SN,

Duong TV, Eto K, Nishikawa K, Matsui M, Orlov NL, Stuart BL, Brown RM, Rowley JJ, Murphy RW, Wang YY, Che J (2018) Large scale phylogenetic analyses provide insights into unrecognized diversity and historical biogeography of Asian leaf-litter frogs, genus *Lepidolax* (Anura: Megophryidae). *Molecular Phylogenetics and Evolution* 124: 162–171. <https://doi.org/10.1016/j.ympev.2018.02.020>
 Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9(8): 772–772. <https://doi.org/10.1038/nmeth.2109>
 Deuti K, Grosjean S, Nicolas V, Vasudevan K, Ohler A (2017) Nomenclatural puzzle in early *Xenophrys* nomina (Anura, Megophryidae) solved with description of two new species from India (Darjeeling hills and Sikkim). *Alytes* 34(1–4): 20–48.
 Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
 Fei L, Hu SQ, Ye CY, Huang YZ (2009) Fauna Sinica. Amphibia Vol. 2 Anura. Science Press, Beijing. [In Chinese]
 Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS One* 2(10): e1109. <https://doi.org/10.1371/journal.pone.0001109>
 Frost DR (2025) Amphibian Species of the World: An Online Reference. Version 6.1. <https://amphibiansoftheworld.amnh.org/> [accessed 15 April 2025]
 Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America* 86(16): 6196–6200. <https://doi.org/10.1073/pnas.86.16.6196>
 Lalronunga S, Zosangliana I, Lalmangaiha K, Lalmingliani E (2020) First record of the Naga Hills Horned Frog, *Megophrys awuh* Mahony, Kamei, Teeling, and Biju (Anura: Megophryidae: Megophryinae), from Mizoram, India. *Reptiles & Amphibians : Conservation and Natural History* 27(3): 472–473. <https://doi.org/10.17161/randa.v27i3.14884>
 Liu S, Hou M, Zhou H, Zuo C, Yin F, Rao D (2024) A New Species of *Herpetoreas* Günther, 1860 (Serpentes: Natricidae) from Yunnan, China. *Diversity* 16(12): 768. <https://doi.org/10.3390/d16120768>
 Liu Z, Chen G, Zhu T, Zeng Z, Lyu Z, Wang J, Messenger K, Greenberg AJ, Guo Z, Yang Z, Shi S, Wang Y (2018) Prevalence of cryptic species in morphologically uniform taxa—Fast speciation and evolutionary radiation in Asian frogs. *Molecular Phylogenetics and Evolution* 127: 723–731. <https://doi.org/10.1016/j.ympev.2018.06.020>
 Luong AM, Pham CT, Nguyen TT, Orlov N, Ziegler T, Nguyen TQ (2022) A new species of *Xenophrys* (Amphibia: Anura: Megophryidae) from Truong Son Range, Vietnam. *Zootaxa* 5150(3): 333–356. <https://doi.org/10.11646/zootaxa.5150.3.2>
 Lyu ZT, Qi S, Wang J, Zhang SY, Zhao J, Zeng ZC, Wang H, Yang JH, Mo YM, Wang YY (2023) Generic classification of Asian horned toads (Anura: Megophryidae: Megophryinae) and monograph of Chinese species. *Zoological Research* 44(2): 380–450. <https://doi.org/10.24272/j.issn.2095-8137.2022.372>
 Mahony S (2011) Two new species of *Megophrys* Kuhl & van Hasselt (Amphibia: Megophryidae), from western Thailand and southern Cambodia. *Zootaxa* 2734(1): 23–39. <https://doi.org/10.11646/zootaxa.2734.1.2>
 Mahony S, Sengupta S, Kamei RG, Biju SD (2011) A new low altitude species of *Megophrys* Kuhl and van Hasselt (Amphibia: Megophryidae), from Assam, Northeast India. *Zootaxa* 3059(1): 36–46. <https://doi.org/10.11646/zootaxa.3059.1.2>

- Mahony S, Teeling EC, Biju SD (2013) Three new species of horned frogs, *Megophrys* (Amphibia: Megophryidae), from northeast India, with a resolution to the identity of *Megophrys boettgeri* populations reported from the region. *Zootaxa* 3722(2): 143–169. <https://doi.org/10.11646/zootaxa.3722.2.2>
- Mahony S, Foley NM, Biju SD, Teeling EC (2017) Evolutionary history of the Asian Horned Frogs (Megophryinae): Integrative approaches to timetree dating in the absence of a fossil record. *Molecular Biology and Evolution* 34(3): 744–771. <https://doi.org/10.1093/molbev/msw267>
- Mahony S, Kamei RG, Teeling EC, Biju SD (2018) Cryptic diversity within the *Megophrys major* species group (Amphibia: Megophryidae) of the Asian Horned Frogs: Phylogenetic perspectives and a taxonomic revision of South Asian taxa, with descriptions of four new species. *Zootaxa* 4523(1): 1–96. <https://doi.org/10.11646/zootaxa.4523.1.1>
- Mahony S, Kamei RG, Teeling EC, Biju SD (2020) Taxonomic review of the Asian Horned Frogs (Amphibia: *Megophrys* Kuhl & Van Hasselt) of Northeast India and Bangladesh previously misidentified as *M. parva* (Boulenger), with descriptions of three new species. *Journal of Natural History* 54(1–4): 119–194. <https://doi.org/10.1080/00222933.2020.1736679>
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE), 2010: 1–8. <https://doi.org/10.1109/GCE.2010.5676129>
- Ohler A, Swan SR, Daltry JC (2002) A recent survey of the amphibian fauna of the Cardomom Mountains, Southwest Cambodia with descriptions of three new species. *The Raffles Bulletin of Zoology* 50(2): 465–482.
- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25(7): 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Rahman MM, Chen JM, Wu YH, Chen HM, Lwin YH, Murphy RW, Li GG, Che J (2020) New country records for three species of frog from Myanmar including two genera (*Nasutixalus* and *Oreolalax*). *Zootaxa* 4742(3): 531–542. <https://doi.org/10.11646/zootaxa.4742.3.7>
- Rambaut A, Drummond AJ (2009) Tracer. Version 1.5. <http://tree.bio.ed.ac.uk/software/tracer/> [accessed 25 January 2021]
- Ronquist F, Teslenko M, Paul VDM, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Saikia B, Sinha B, Thakur M, Kharkongor IJ (2019) *Megophrys maosonensis* Bourret, 1937 (Anura: Megophryidae) – A New India Record from Talley Valley Wildlife Sanctuary, Arunachal Pradesh, India. *Records of the Zoological Survey of India*: 456–462. <https://doi.org/10.26515/rzsi/v119/i4/2019/142151>
- Saikia B, Sinha B, Shabnam A, Kharkongor IJ, Dinesh KP (2024) On a new species of *Xenophrys* (Anura: Megophryidae) from Tale Wildlife Sanctuary, Arunachal Pradesh, India with comments on the earlier erroneous report as *X. maosonensis* (Bourret, 1937). *Records of the Zoological Survey of India* 124: 21–40. <https://doi.org/10.26515/rzsi/v119/i4/2019/142151>
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Shi SC, Wang B, Zhu WB, Fu L, Jiang W, Li DH, Jiang JP (2020) *Megophrys periosa* (Mahony, Kamei, Teeling, and Biju 2018) was first recorded in Yunnan province, China with description of its tadpole. *Dongwuxue Zazhi* 55(6): 730–740.
- Shu G, Li K, Wu Y, Liu Q, He Z, Li L, Zhang H, Guo P (2023) A new species of *Xenophrys* (Amphibia, Anura, Megophryidae) from southern Tibet, China. *ZooKeys* 1182: 307–329. <https://doi.org/10.3897/zookeys.1182.106828>
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* (Oxford, England) 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Stuart BL, Chuaynkern Y, Chan-ard T, Inger RF (2006) Three new species of frogs and a new tadpole from eastern Thailand. *Fieldiana. Zoology* 2006(111): 1–19. [https://doi.org/10.3158/0015-0754\(2006\)187\[1:TNSOFA\]2.0.CO;2](https://doi.org/10.3158/0015-0754(2006)187[1:TNSOFA]2.0.CO;2)
- Sun YB (2018) FasParser2: A graphical platform for batch manipulation of tremendous amount of sequence data. *Bioinformatics* (Oxford, England) 34(14): 2493–2495. <https://doi.org/10.1093/bioinformatics/bty126>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Wang K, Yu ZB, Wu YH, Hou SB, Gao W, Duan SH, Xiong Y, Jiang YF, Liu KC, Li JH, Yang Q, Huang XY, Feng YG, Duan ZP, Yin FW, Shu SQ, Wu YY, Che J (2024) Herpetofauna diversity of the Gaoligong Mountain in China: Checklist, zoogeography, and conservation. *Zoological Research. Diversity and Conservation* 1(3): 169–179. <https://doi.org/10.24272/j.issn.2097-3772.2024.003>
- Wu YH, Hou SB, Yuan ZY, Jiang K, Huang RY, Wang K, Liu Q, Yu ZB, Zhao HP, Zhang BL, Chen JM, Wang LJ, Stuart BL, Chambers EA, Wang YF, Gao W, Zou DH, Yan F, Zhao GG, Fu ZX, Wang SN, Jiang M, Zhang L, Ren JL, Wu YY, Zhang LY, Yang DC, Jin JQ, Yin TT, Li JT, Zhao WG, Murphy RW, Huang S, Guo P, Zhang YP, Che J (2023) DNA barcoding of Chinese snakes reveals hidden diversity and conservation needs. *Molecular Ecology Resources* 23(5): 1124–1141. <https://doi.org/10.1111/1755-0998.13784>
- Wu YH, Yu ZB, Chen JM, Kilunda FK, Zhang DC, Zuo CS, Zuo AR, Duan ZP, Che J (2024) A field survey on the genus *Xenophrys* (Amphibia, Megophryidae) confirms underestimated diversity in the Gaoligong Mountains, with the description of a new species. *Zoosystematics and Evolution* 100(3): 1041–1052. <https://doi.org/10.3897/zse.100.127635>
- Wu YH, Yu ZB, Yang SP, Duan ZP, Zuo AR, Zhang DC, Kilunda FK, Murphy RW, Che J (2025) Morphological and molecular evidence for a new species of the genus *Leptobrachella* (Anura, Megophryidae) from Gaoligong Mountain Range, Yunnan, China. *Zoosystematics and Evolution* 101(2): 449–463. <https://doi.org/10.3897/zse.101.135560>
- Xu YH, Yang DC, Gong YA, Ouyang KC, Weng SY, Deng JD, Huang S, Peng LF (2024) A new species of the genus *Hebius* (Squamata, Natricidae) from Yunnan, China. *ZooKeys* 1206: 255–274. <https://doi.org/10.3897/zookeys.1206.123841>
- Yang J, Chan BPL (2018) A new phytotelm-breeding treefrog of the genus *Nasutixalus* (Rhacophoridae) from western Yunnan of China. *Zootaxa* 4388: 191–206. <https://doi.org/10.11646/zootaxa.4388.2.3>
- Yang J, Zeng ZC, Wang Y (2018) Description of two new sympatric species of the genus *Leptolalax* (Anura: Megophryidae) from western Yunnan of China. *PeerJ* 6(e4586): 1–32. <https://doi.org/10.7717/peerj.4586>
- Yu ZB, Kilunda FK, Wang K, Cao YY, Wu CL, Duan ZP, Zuo CS, Zhang DC, Wu YH, Che J (2024) The first discovery of *Polypedates teraiensis* (Dubois, 1987) (Rhacophoridae, Anura) in China. *Biodiversity Data Journal* 12: e127029. <https://doi.org/10.3897/BDJ.12.e127029>